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CLEAVAGE AND MESENCHYME FORMATION IN TOXOPNEUSTES VARIEGATUS.

MARY J. GUTHRIE AND HOPE HIBBARD,
BRYN MAWR COLLEGE.

This investigation was undertaken for the purpose of determining the origin of the primary mesenchyme in Echinoidea. Although numerous researches have been carried out on the early stages in the development of echinoids, the question of the origin of the mesenchyme has never been definitely settled.

MacBride,[1] writing of *Echinus esculentus*, states that "this mesenchyme originates, almost certainly, from the descendants of the smaller micromeres." The figures which Boveri (1901) gives of the eggs of *Strongylocentrotus lividus* indicate that this is true, and it is believed that unmistakable evidence is herein presented that in *Toxopneustes variegatus* (*Lytechinus variegatus*) the mesenchyme arises from the micromeres.

MATERIAL AND METHODS.

The material on which this investigation was carried out was obtained by Dr. D. H. Tennent, during the summer of 1908, in the course of work at the United States Fisheries Biological Station, Beaufort, N. C.

The segmenting eggs were fixed in either picro-acetic or sublimate-acetic. In both cases the solutions were two per cent. acetic acid. The observations recorded here were made on whole embryos stained with borax carmine and safranin, and on sectioned material stained with borax carmine or iron hæmatoxylin.

In addition to the above material, Dr. Tennent lent camera

[1] "Text-book of Embryology," p. 507.

lucida sketches of the cleavage stages which he made from living eggs. While it is possible to make out these stages in the preserved specimens, they are much clearer in the living eggs; and his sketches have been used to illustrate the segmentation changes.

Early Cleavage.

The *Toxopneustes* egg has been found especially favorable for many kinds of study because of its transparency and beautiful clearness of structure, when fixed as well as while alive.

As this paper deals with a definitely limited phase of development it seems best not to include a detailed description of the egg, or its jelly layer, or of the manner of formation of the fertilization membrane, but to pass at once to the consideration of cleavage.

The first cleavage of the *Toxopneustes* egg is vertical and results in two equal cells being formed about forty minutes after insemination (Figs. 1, 2, 3 and 4). Tennent (1911) gave the characteristic rhythm for *Toxopneustes* cleavage at 28° C., and the time intervals indicated are from his paper. This rhythm may be altered by changes in temperature as has been shown for example by Loeb and Chamberlain (1915) who worked with *Arbacia* eggs. They found a close correlation between the temperature of the water in which the eggs were kept and the period of time elapsing between insemination and the first cleavage, and were, therefore, able to calculate the temperature coefficient for cell division at varying temperatures.

The second cleavage, about one hour and ten minutes after insemination, is also vertical, and divides the egg into four equal cells which are shown in polar view in Fig. 5. After one hour and thirty-five minutes, the third cleavage furrow coming in horizontally divides the egg into eight equal cells (Figs. 6 and 7). This is the last cleavage in which all of the cells are equally divided.

A twelve cell stage is formed by a horizontal division which cuts off four small cells, the first set of micromeres, at the vegetal pole. Practically coincident with this, the four cells at the animal pole are divided by vertical furrows into eight. In the sixteen cell stage there are therefore, three different kinds of

cells as regards size, these being respectively, the small macromeres, a group of eight medium-sized cells at the animal pole; the micrometers, four small cells at the vegetal pole; and the large macromeres, four large cells lying between the two groups just mentioned. These are shown as seen from the side in Fig. 8 and as seen from the vegetal pole in Fig. 9. It is one hour and fifty minutes after the addition of the sperm when this stage is complete.

From this time on, all of the cells do not divide simultaneously. Two hours and ten minutes after insemination the four large macromeres are divided vertically into eight, and the eight small macromeres are divided vertically into sixteen. About ten minutes later the micromeres are cut horizontally into eight, but this is an unequal division, the four smaller micromeres lying nearer the vegetal pole (Fig. 10). The completed thirty-two-celled embryo is shown from the side in Fig. 11, and the crowding in of the micromeres can be noticed at this stage.

The next furrow cuts the lower set of eight large macromeres horizontally, and twenty minutes later, or two hours and fifty-five minutes after insemination, vertical cleavages divide the upper set of large macromeres into sixteen, and the sixteen small macromeres into thirty-two. After three hours and five minutes, the upper micromeres are divided vertically into eight, the four micromeres referred to above as lying nearer the vegetal pole, now being crowded back and partially concealed by this ring of eight cells. Five minutes later the lowest set of macromeres is divided by vertical cleavages into sixteen (Fig. 12). Twelve of the seventy-six cells of this stage are micromeres, the later development of which will be taken up presently.

The following table (page 142) shows in a diagrammatic way, the sequence of cleavage in the *Toxopneustes* egg. The time indicated is the elapsed time after insemination.

Theel's (1892) account for *Echinocyamus pusillus* differs from this in that he describes the micromeres as being cut off at the animal pole. According to his description the sixteen-cell stage is completed in about two hours and twenty minutes and is accomplished as follows: "First the four upper segments are divided by a horizontal plane into four small cells or micromeres and

TABLE SHOWING SEQUENCE OF CLEAVAGE.

Time.	40 m.	1 h. 10 m.	1 h. 35 m.	1 h. 50 m.	1 h. 50 m.	2 h. 10 m.	2 h. 20 m.	2 h. 35 m.	2 h. 55 m.	2 h. 55 m.	3 h. 5 m.	3 h. 10 m.
Small macromeres			4	4	8	16	16	16	32	32	32	32
Blastomeres	2	4										
Large macromeres			4	4	4	8	8	16	16	24	24	32
Micromeres				4	4	4	4	4	4	4	8	8
							4	4	4	4	4	4
Total cells	2	4	8	12	16	28	32	40	56	64	68	76

[1] It will be noticed that there are two columns headed 1 h. 50 m. and two headed 2 h. 55 m. This is for the purpose of indicating that the formation of the micromeres is completed before complete division of the four small macromeres, about one hour and fifty minutes after insemination, and that the division of the sixteen small macromeres is completed before that of the upper eight large macromeres, two hours and fifty-five minutes after insemination. The duration of an actual twelve cell and of an actual fifty-six-cell stage is short.

four large ones. Immediately after this segmentation or, though more seldom, before it is accomplished, the four lower segments are divided by a vertical plane into eight almost equal spheres, which become arranged in a more or less irregular manner in two rows."

Further, in *Echinocyamus*, two hours and thirty-five minutes after fecundation, the large macromeres are divided vertically, the small macromeres are cut horizontally, and after fifteen or twenty minutes "the four micromeres at the animal pole become parted by horizontal planes into eight, four of which are smaller, contain a clearer protoplasm and, as they lie in the same plane, constitute the animal pole itself; the four remaining ones have their place somewhat outside and below." It will be noticed that the small macromeres are divided horizontally in *Echinocyamus*, while in *Toxopneustes* the division is vertical.

Theel fell into error concerning the micromeres probably because he assumed that they were homologous with the micromeres of flatworms, annelids and molluscs. Boveri ('01) in his study of the egg of *Strongylocentrotus lividus*, demonstrated that it is at the vegetal pole that the micromeres are separated at the twelve-cell stage.

FORMATION OF MESENCHYME.

Very early in the development of the *Toxopneustes* blastula the cells which are to give rise to the future mesenchyme can

be distinguished. With the cutting off of the micromeres there is separated from the rest of the embryo that protoplasm which later goes to form the mesenchyme. These cells, or cells derived from them, must pass from the wall of the blastula into its cavity; and as early as the sixteen-celled stage some of the first four micromeres exhibit this tendency to migrate inward. Sections passing through the poles of a sixteen-celled stage show the micromeres visibly crowded. Figs. 13 and 14 are sections of two different individuals in this stage, and the micromeres at the vegetal pole are projecting slightly into the blastocœle with their inner ends bulging as a result of pressure from the adjacent cells in the wall. The difference in the number of the micromeres in the two figures is due to the planes of the sections. Fig. 13 is cut parallel to the short axis of the group of micromeres, while Fig. 14 is cut parallel to the long axis of the group.

A later stage is shown in Fig. 15 which is a somewhat oblique section through the micromeres. In this case the micromeres have multiplied and the process of pushing in has continued until one cell of the particular section in question has lost all connection with the surface.

Figs. 16*a*, 16*b* and 16*c* represent three serial sections of one specimen which is slightly older than the preceding one. Here the migration is quite evident. From this time on the micromeres are not readily distinguishable by their small size as heretofore, since by an unequal rate of division, all the cells have become more nearly the same size. The fact that in earlier stages it was the micromeres, and they alone, which passed inward, and the fact that throughout the stages the migration is restricted to a limited area in the wall, afford proof that in the later stages the mass of cells which has passed inward represents the offspring of the original micromeres.

A more pronounced wandering in of cells is shown in Figs. 17*a*, 17*b* and 17*c*—serial sections of one embryo. Here, certainly, pressure from without has ceased to operate as the cause of migration, for the wandering cells are free to a great degree from contact with cells of the wall.

There have been various theories of the cause of the separation of the mesenchyme cells from the wall of the blastula in other

forms. Grave, in *Ophiura brevispina*, describes the mesenchyme cells as being pushed or squeezed inward by the coercive pressure of the organism as a whole, though this is not the whole story of the process. MacBride describes the migration of mesenchyme cells in *Asterias vulgaris* thus: "The 'wandering' of these cells seems to be effected by their emitting long filamentous pseudopodia which span the blastocœle, and along these strands the body of the cell glides like a drop of dew on a spider's web."

Serial sections through an embryo figured in 18*a*, 18*b* and 18*c*, show the mesenchyme cells much more numerous, and those most recently budded off are compactly pressed together. In Fig. 19 the cells have multiplied still more and the mesenchyme lies close to that part of the wall from which it arose. Fig. 20, a stage still later, shows the mass of mesenchyme cells much larger but still compact. In this set of material this condition was found one hour and forty minutes before the first indication of gastrulation, namely, the flattening of the vegetal pole.

When the process of gastrulation begins, the mesenchyme cells migrate laterally and become arranged in a ring in the blastocœle about the polar axis of the embryo. The invagination of the archenteron proceeds from the vegetal pole toward the space unoccupied by mesenchyme. Fig. 21 is a very early gastrula with the mesenchyme cells lateral to the invaginating archenteron. A horizontal section through an early gastrula in approximately the same stage as Fig. 21 is shown in Fig. 22. The ring of mesenchyme is distinctly visible around the archenteron. A later gastrula is shown in Fig. 23 in which the secondary mesenchyme is being proliferated from the inner end of the archenteron. The primary mesenchyme may be seen at the sides of the archenteron. This specimen was somewhat distorted in sectioning and for comparison, Fig. 24, drawn from a camera lucida sketch of the living gastrula, has been inserted.

Since the publication of Theel's paper, in which the literature on the subject up to 1892 was discussed, the origin of the mesenchyme has been described for several echinoderms. Like the earlier accounts, these, with the exception of those of Boveri and MacBride, deal rather with the general question as to whether the mesenchyme arises in the blastula at the vegetal pole, or in

the gastrula from the archenteron, either during or after its invagination, than with the idea of associating the primary mesenchyme with an early differentiation in the segmenting egg.

The following table gives a resumé of the more recent literature, with the inclusion of Bury's observations of earlier date on *Antedon*.

TABLE SHOWING TIME OF MESENCHYME FORMATION.

Date.	Author.	Form.	Time of Mesenchyme Formation.
		ASTEROIDEA.	
1892	Field.	*Asterias vulgaris.*	Blastula, through gastrulation.
1896	MacBride.	*Asterina gibbosa.*	After complete gastrulation.
1902	Masterman.	*Cribrella oculata.*	After complete gastrulation.
1912	Gemmill.	*Solaster endeca.*	As morula changes to blastula.
1914	Gemmill.	*Asterias rubens.*	Late gastrula.
		OPHIUROIDEA.	
1897	MacBride.	*Ophiothrix fragilis.*	Blastula.
1916	Grave.	*Ophiura brevispina.*	Blastula.
		ECHINOIDEA.	
1901	Boveri.	*Strongylocentrotus lividus.*	Blastula.
1903	MacBride.	*Echinus esculentus.*	Blastula.
		" *miliaris.*	Blastula.
		" *acutus.*	Blastula.
1904	Schmidt.	*Echinus microtuberculatus.*	Blastula.
1914	MacBride.	*Echinocardium cordatum.*	Blastula.
1914	Tennent.	*Cidaris tribuloides.*	Gastrula.
		CRINOIDEA AND HOLOTHUROIDEA.	
1898	Clark.	*Synapta vivipara.*	Gastrula.
1909	Edwards.	*Holothuria floridana*	Blastula at beginning gastrulation.
1888	Bury.	*Antedon rosacea.*	Gastrula.
1892	Seeliger.	" "	Gastrula.

It will be seen that among the Asteroidea there are some differences between various forms in their methods of mesenchyme formation. Field (1892) described these cells in *Asterias vulgaris* as being given off from the developing archenteron even from the beginning of its invagination. Gemmill (1914) found no mesenchyme cells in the normal larvæ of *Asterias rubens* until gastrulation was well advanced, when they appeared at the end of the archenteron. MacBride (1896) found in *Asterina gibbosa* that no mesenchyme was formed until after gastrulation was complete. In connection with these accounts it is interesting to note the results of Herbst (1896) who experimentally induced larvæ of *Asterias glacialis*, which normally produce mesenchyme only

after gastrulation, to form large numbers of mesenchyme cells while still in the blastula stage.

In contrast with these processes, Masterman (1902) in *Cribrella oculata*, and Gemmill (1912) in *Solaster endeca*, have described a very different series of events. In *Solaster* the cleavage results in a solid morula of nearly equal cells. The outside of this mass then becomes wrinkled by the appearance of convoluted furrows which so increase the surface that the cells inside can become arranged in a single layer, leaving a flattened, irregular cavity, the blastocœle. The furrows gradually disappear as the cells over the entire surface become longer and narrower until the larva becomes smooth once more. The initiation of the process of gastrulation also aids in flattening the surface. During these changes some wandering cells fail to arrange themselves in a layer with the others and are left behind in the cavity of the blastula. These are the primary mesenchyme cells. In *Cribrella* similar phenomena occur, but instead of being left behind as the morula changes to a blastula, the mesenchyme cells are budded off after invagination, from the "hypoblast" into the blastocœle, in the manner of the more typical Asteroidea. In addition to this, cells morphologically similar to mesenchyme cells are budded off from the other side of the "hypoblast" into the archenteron, and were called "hypenchyme" by Masterman.

Even fewer species of the Ophiuroidea have been investigated on this point. Grave (1916) in *Ophiura brevispina* found a large number of mesenchyme cells in the blastocœle of early stages. MacBride (1897) observed mesenchyme in blastulæ of *Ophiothrix fragilis* between four and five hours after fertilization, while gastrulation did not begin until about the eighteenth hour.

The majority of Echinoidea show early mesenchyme formation. MacBride (1903) figured blastulæ of *Echinus miliaris*, *E. acutus* and *E. esculentus* with mesenchyme forming at one end, which was, in some cases, slightly flattened. In *E. esculentus* he found about fifty mesenchyme cells which were probably derived from the micromeres in the blastula. In *Echinocardium cordatum* (MacBride 1914) mesenchyme is found at the vegetal pole long before any signs of gastrulation. MacBride was unable to confirm the observations of Fleischman (1888) on this form with

regard to the four arch-mesenchyme cells which Fleischman found. Schmidt (1904) observed mesenchyme cells in the blastula of *Echinus microtuberculatus*. They were present at the seventeen-hour stage and continued to be formed until the twenty-four hour stage. Boveri (1901) in his work on *Strongylocentrotus lividus*, found in the unsegmented egg a portion at the vegetal pole that was unpigmented. This region gave rise to the micromeres, and they in turn to mesenchyme in the blastula stage. The lack of pigment in these cells made their development easy to follow.

Tennent (1911) noted the presence of four mesenchyme cells in the blastocœle of the *Toxopneustes* blastula about eight hours after the fertilization of the egg and about an hour before the beginning of gastrulation. An exception to the typical echinoid condition is shown by *Cidaris tribuloides* (Tennent, 1914), in which the formation of the mesenchyme is delayed and is given off from the inner end of the archenteron in the gastrula.

To our knowledge of mesenchyme formation in the Holothuroidea the observations of Clark (1898) that in the blastula of *Synapta* there is no evidence of mesenchyme cells, but that they arise later, wandering in from the whole surface of the archenteron, and of Edwards (1909) that in *Holothuria floridana* the mesenchyme begins by cell-proliferation at the vegetative pole of the blastula, while at the same time gastrulation takes place, confirm the earlier evidence of Korschelt, Selenka and Ludwig to the effect that in the Holothuroidea the time of mesenchyme formation varies.

Bury (1888) and Seeliger (1892) from their study of the development of *Antedon rosacea* agree that the formation of the mesenchyme does not begin until after the beginning of the processes of gastrulation.

From these accounts it may be seen that, with the exception of Boveri's work on *Strongylocentrotus* and MacBride's work on *Echinus esculentus*, little attempt has been made to trace the origin of the mesenchyme cells further back than to the approximate point in the larva from which they arise. If formed early they come from the vegetal pole of the blastula, if late from the archenteron. Theel makes the statement that "the mesenchyme

arises earliest, with very few exceptions, in those groups of echinoderms in which the larvæ have their skeleton earliest developed." Since one of the functions of the primary mesenchyme is the formation of the skeletal rods of the larvæ the reason for this correlation is evident.

In this paper it has been shown that the primary mesenchyme in *Toxopneustes* comes from the micromeres. Driesch (1900) has shown by experiments on fragments of blastulæ, that after the micromeres have been formed, the cells at the opposite pole of the larva are unable to form mesenchyme. The micromeres arise at the vegetal pole of the segmenting egg, but cannot be considered as either ectoderm or endoderm. The primary mesenchyme is the result of an earlier differentiation than either ectoderm or endoderm.

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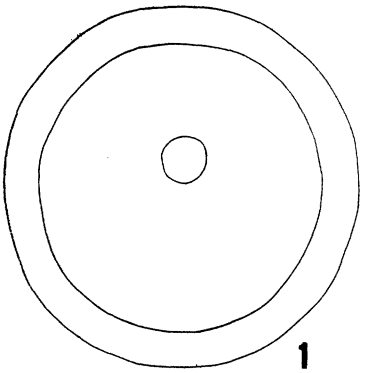
DESCRIPTION OF PLATES.

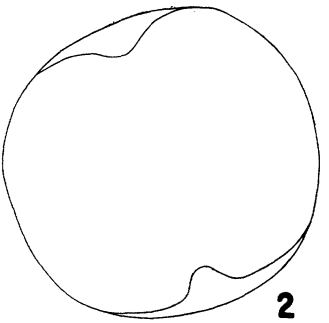
Figs. 1 to 12 and Fig. 24 were made from camera lucida sketches of living eggs. A pantograph was used to enlarge them two and one half times, and this gave a magnification of 1,050 diameters. They have been reduced one half in reproduction so that the magnification is now 525 diameters.

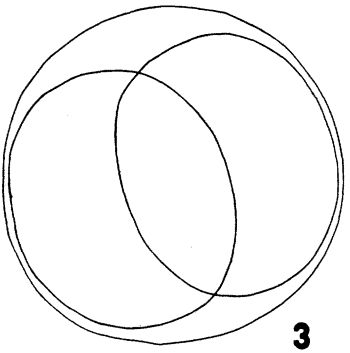
Figs. 13 to 23 were made from camera lucida drawings of mounted sections which were enlarged two and one half times and reduced one half giving a final magnification of 725 diameters.

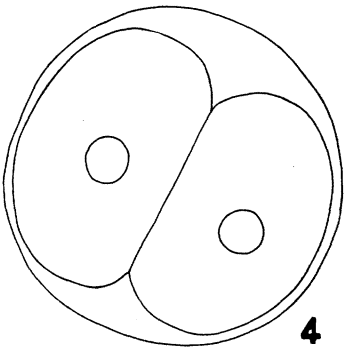
EXPLANATION OF PLATE I.

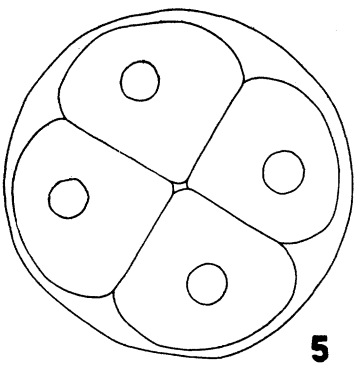
FIG. 1. Fertilized egg.
FIG. 2. Incomplete 2-celled stage.
FIGS. 3 and 4. 2-celled stage.
FIG. 5. Polar view of 4-celled stage.
FIG. 6. Incomplete 8-celled stage.

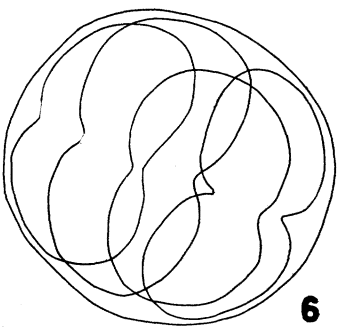












GUTHRIE AND HIBBARD.

EXPLANATION OF PLATE II.

FIG. 7. Side view of 8-celled stage.

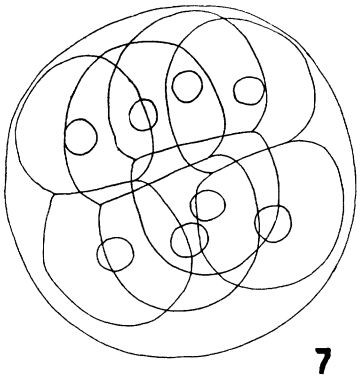
FIG. 8. Side view of 16-celled stage showing the micromeres cut off at the vegetal pole.

FIG. 9. View of 16-celled stage from vegetal pole.

FIG. 10. View of 32-celled stage from vegetal pole showing second set of micromeres.

FIG. 11. Side view of 32-celled stage.

FIG. 12. Polar view of a 76-celled stage showing the twelve micromeres.



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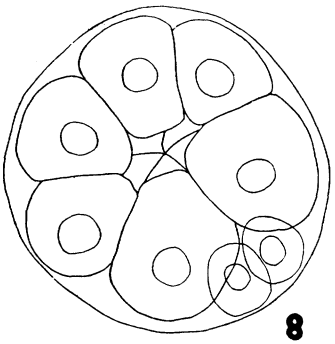

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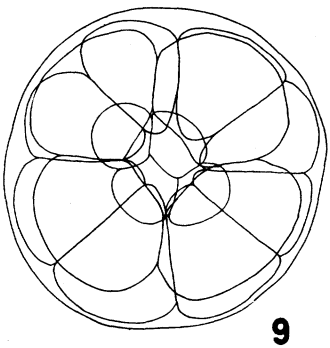

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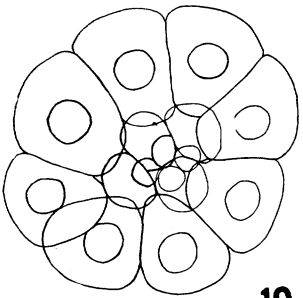

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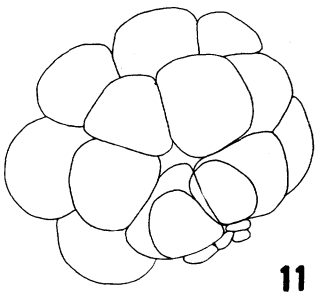

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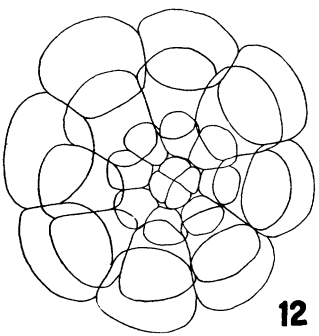


12

Explanation of Plate III.

Fig. 13. Section through a 16-celled stage passing through the short axis of the group of micromeres.

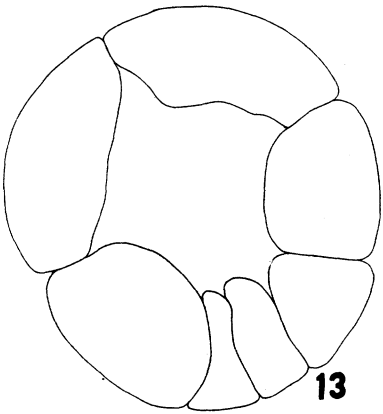
Fig. 14. Section through a 16-celled stage passing through the long axis of the group of micromeres.

Fig. 15. Section of slightly older stage showing multiplication of micromeres.

Figs. 16*a*, 16*b*, 16*c*. Serial sections of an older embryo showing migration of micromeres.

Figs. 17*a*, 17*b*, 17*c*. Serial sections of a still older embryo showing further migration of micromeres.

Figs. 18*a*, 18*b*. Two of a series of sections showing more numerous mesenchyme cells (micromeres).



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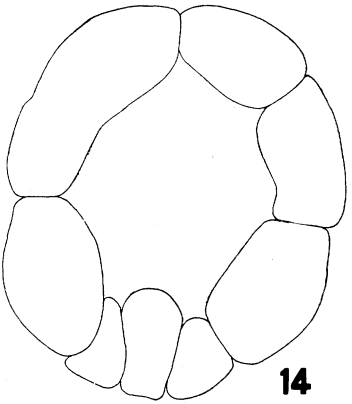

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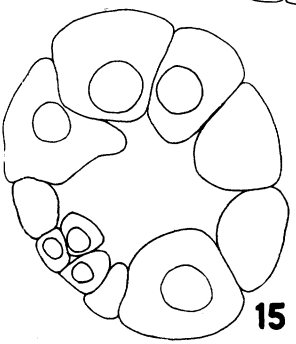

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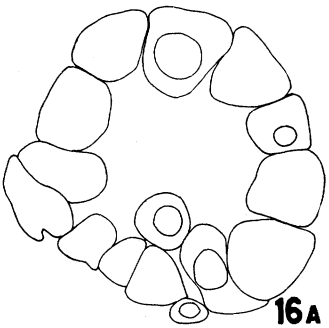
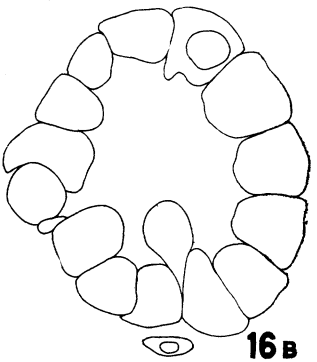
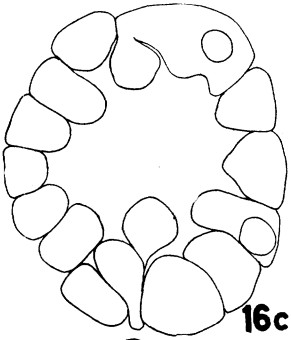


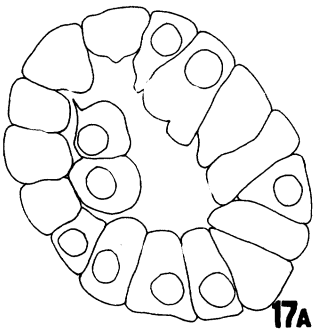
16A



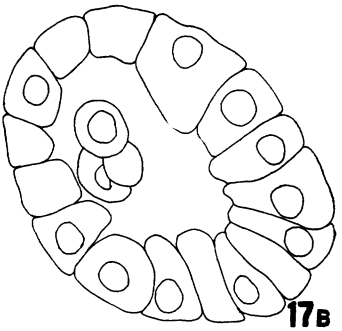
16B



16C



17A



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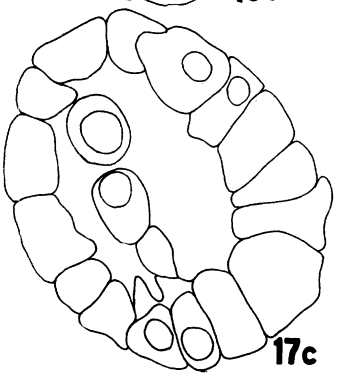

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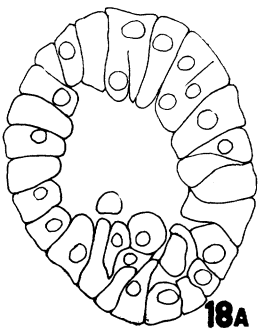
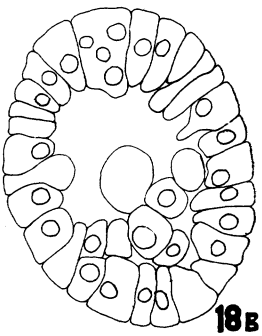


18A



18B

EXPLANATION OF PLATE IV.

FIG. 18*c*. Third section in the same series as Figs. 18*a* and 18*b* showing more numerous and compact mesenchyme cells.

FIG. 19. Section through slightly older embryo showing continued multiplication of mesenchyme cells.

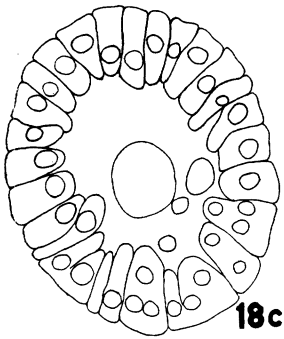
FIG. 20. Section through a later stage than Fig. 19.

FIG. 21. Vertical section through an early gastrula.

FIG. 22. Horizontal section of same stage as Fig. 21.

FIG. 23. Vertical section of older gastrula.

FIG. 24. Entire living gastrula.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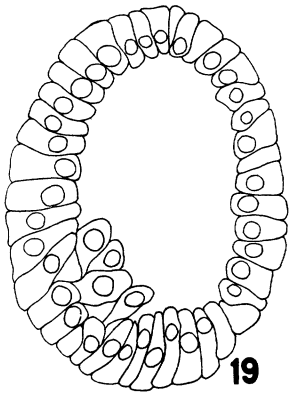
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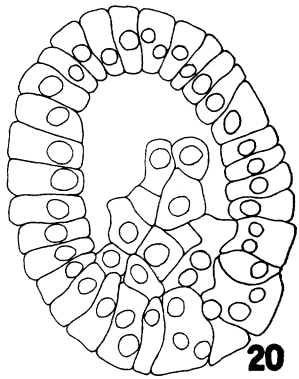
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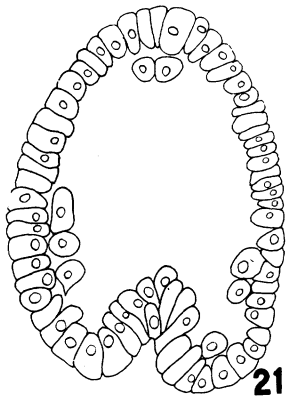
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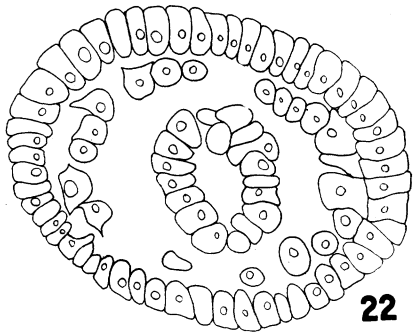
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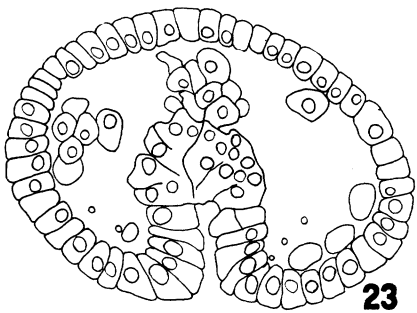
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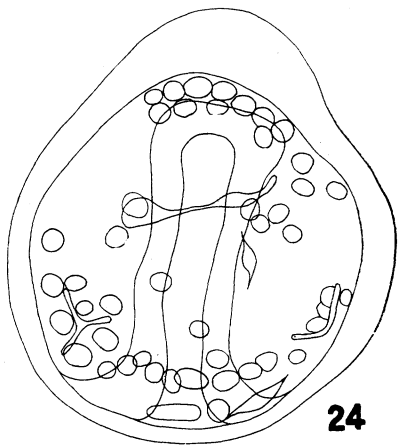
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